GEIS Gazette

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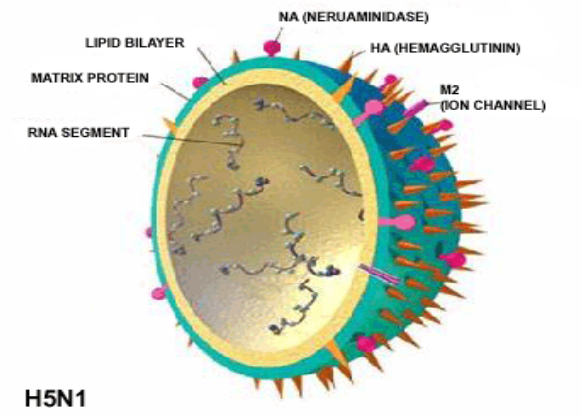
AVIAN INFLUENZA: THE WAY FORWARD

Samson Limbaso

Avian Influenza (AI) is a viral disease affecting the respiratory, digestive and nervous system of many bird species. It can occur in most species of birds both domestic and wild. It was first described in 1878 in Italy as serious disease of chicken known as Fowl Plague. In 1955 it was determined that Fowl Plague Virus is one of the Influenza viruses.

AI is a member of the Orthomyxoviridae Virus family. The virus particle has an envelope with glycoprotein projections which have hemagglutinating and neuraminidase activity. These two surface antigens, hemagglutinin (HA) and neuraminidase (NA) form the basis of description for the serologic identity of the Influenza Viruses using the letters H and N with the appropriate numbers in the virus designation. There are now 15 hemagglutinin (H1 – H15) and 9 neuraminidase (N1 – N9) antigens described among the Type A Influenza Viruses. The type designation (A, B, or C) is based upon the antigenic character of the M protein of the virus envelope and the nucleoprotein within the virus particle. All Influenza Viruses affecting domestic animals i.e. equines, swine and avian belong to Type A and it is the most common type producing serious epidemics in humans. The other types i.e. B and C do not affect domestic animals.



A particular strain of Influenza virus is named according to its type (A, B, or C), host of origin, geographical location, strain number, year of isolation and antigenic subtype e.g. if Influenza A, subtype H7N3 was isolated from chickens in a certain geographical location, during a 2003 outbreak, the strain would be named: A/Chicken/geographical location/1/03(H7N3).

Natural hosts of AI include the domestic fowl, ducks, geese, turkeys, guinea fowl, quail, and pheasants. The source of infection has not been ascertained but it could be due to direct or indirect contact with migratory waterfowl. The virus spreads rapidly in flocks by direct contact and is shed in faeces and nasal and other ocular discharges. Clinical symptoms vary in birds depending on the virulence of the virus and species affected and ranges from sudden death to signs like depression, loss of appetite, cyanosis of wattle and combs, respiratory distress and diarrhea.

Although Avian Influenza A Viruses do not usually infect humans, several instances of human infections and outbreaks of AI have been reported since 1997**:**

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Walter Reed Project
GEIS Department
P.O. Box 606
Nairobi 00621
Tel: 254-20-2729303
Fax: 254-20-2714592

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- H5N1, Hong Kong, 1997: Avian Influenza A (H5N1) occurred in both poultry and humans. This was the first time an Avian Influenza Virus had ever been found to transmit directly from birds to humans. 18 people were hospitalized and six of them died. To control the outbreak, 1.5 million chickens were killed. It was determined that the virus spread primarily from birds to humans, though rare person-to-person infection was noted.
- H9N2, China and Hong Kong, 1999: Avian Influenza A H9N2 illness was confirmed in two children.
- H7N2, Virginia, 2002: Following an outbreak of H7N2 among poultry in the Shenandoah Valley poultry production area, one person was found to have serologic evidence of infection with H7N2.
- H5N1, China and Hong Kong, 2003: Two cases of Avian Influenza A (H5N1) infection occurred among members of a Hong Kong family that had traveled to China.
- H7N7, Netherlands, 2003: The Netherlands reported outbreaks of Influenza A (H7N7) in poultry on several farms. Later, infections were reported among pigs and humans. In total 89 people were confirmed to have H7N7 Influenza virus infection associated with this outbreak.

The above are just a few outbreaks which continue up to today. Symptoms in humans include typical flu-like symptoms i.e. fever, sore throat, cough and muscle aches. Other symptoms include eye infections, pneumonia, acute respiratory distress and other severe and life threatening complications may occur.

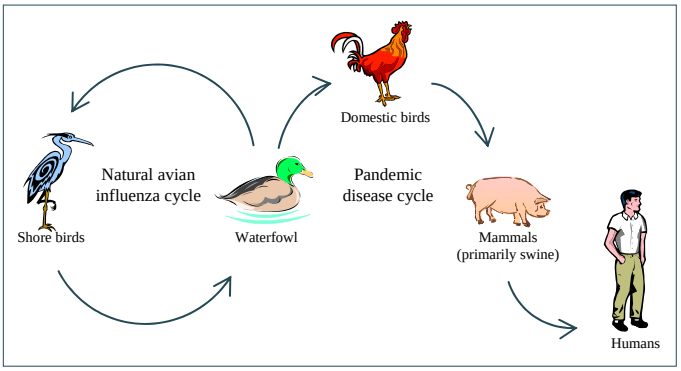
Avian Influenza is a viral disease affecting the respiratory, digestive and nervous system of many bird species

In Africa, South Africa has borne the brunt of AI greatly. It supplies about 70 percent of the world's ostrich meat, producing 1,000 tons a year. In August 2004, the Avian Influenza Virus killed 1,500 ostriches on two farms in the Eastern Cape prompting government to order the slaughter of more than 30,000 birds in a bid to contain and control the disease. A diagnosis of H5N2 virus was made. Many countries imposed bans on poultry products from the country. This clearly demonstrates the ability of the virus to cause economic havoc in affected countries and it takes great effort and time to undo the damage.

The virus is a threat to human and animal health, food supply, puts a strain on public health resources and can have great economic consequences.

The virus also has a high potential for causing a pandemic. This is because it can mutate, cause illness in humans and there is little immunity in humans to avian strains of influenza which would mean that control would be difficult to implement. The influenza strain H5N1 is of particular concern.

From the above it is clear that the virus is a threat to human and animal health, food supply, puts a strain on public health resources involved in its control and prevention and can have great economic consequences.



Cycle of Avian Influenza Virus in Animals and Humans

To combat this growing threat and be ready for any eventuality, there is need for the following**:**

- Need for regional laboratories which can test both bird and human specimens
- Provision of periodic updates on Avian Influenza activity in different regions
- Laboratory and field surveillance which permits implementation of control measures if needed
- Vaccine trials are underway for the H5N1 strain, but this is in its early phase.

There is need for all countries to be on the lookout for Avian Influenza Virus activity. Defense is the best offence. The growing threat presents great opportunities in research in the field of epidemiology, vaccine development, and diagnostics. The many disciplines involved in Avian Influenza control and prevention presents opportunities for cooperation and a greater understanding of the virus and its potential threats.

Safety Practices in the Laboratory

Valerie Oundo

Laboratory personnel working with infectious agents are subject to laboratory acquired infections through accidents or unrecognized incidents. The degree of hazard depends upon the virulence of the biological agent concerned and host resistance. Laboratory-acquired infections occur when micro-organisms are inadvertently ingested, inhaled, or introduced into the tissues.

Biosafety levels determine the nature of the pathogens or reagents that a laboratory can handle safely and the relative risk to the personnel working there. Biosafety Level (BSL) is divided into three categories: BSL-1 is appropriate for working with micro-organisms that are not known to cause disease in healthy human humans, BSL-2 are designed to maximize safe working conditions for laboratorians working with agents of moderate risk to personnel and the environment, and BSL-3 is suitable for work with infectious agents which may cause serious or potentially lethal diseases as a result of exposure by the inhalation route

Most common laboratories have BSL-2 requirements which include:

- Laboratory personnel should have specific training in handling pathogenic agents and are directed by competent scientists;
- Access to the laboratory is limited when work is being conducted;
- Extreme precautions are taken with contaminated sharp items;
- Certain procedures in which infectious aerosols or splashes may be created are conducted using protective clothing and equipment.

Standard Microbiological Safety Practices are those that apply to all microbiology laboratories regardless of biosafety level. These are:

Limiting access to laboratory

Biohazard signs or stickers should be posted near all laboratory doors and on all equipment (incubators, hoods, refrigerators, freezers) used for laboratory work. Children and pets are not allowed in laboratory areas. All laboratories should be locked when not in use. All freezers and refrigerators located in corridors should be locked.

Hand washing

Each laboratory should contain a sink for hand washing. Frequent hand washing is one of the most effective procedures for avoiding laboratory acquired infections. Hands should be washed with an appropriate germicidal soap before exiting the laboratory or after handling infectious materials.

Eating

Eating, drinking, and smoking are not permitted in the work areas. Food must be stored and eaten outside of the work area in designated areas used for that purpose only. Do not lay personal articles such as handbags or eyeglasses on the workstations.

Mouth pipetting

Mouth pipetting should be strictly prohibited in the laboratory. Rubber bulbs or mechanical devices should be used.

Sharps

A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Dispose of sharps in designated containers. To minimize finger sticks, used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Broken glassware should not be handled directly by hand but should be removed by mechanical means such as a brush and dustpan, tongs, or forceps.

Aerosols

Perform all procedures carefully to minimize the creation of splashes or aerosols. Techniques that tend to produce aerosols should be avoided. Cool inoculating wires and loops by holding them still in the air for 5 to 10 seconds before touching colonies or clinical material. Loops containing infectious material should be dried in the hot air above the burner before flaming. Vortexing and centrifugation should be done in closed containers. Gauze should be used to remove the tops on blood specimens and should be placed around the top of blood culture bottles to minimize aerosol production during removal of the needle.

All body fluids should be centrifuged in carriers with safety caps only.

When procedures with a high potential for creating infectious aerosols are conducted or when there is a risk of splashing or spraying the face with infectious or other hazardous materials, laboratory work should be conducted in a safety cabinet or with face protection (goggles, mask, face shield or other splatter guards). Procedures that pose a risk may include

centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs. Face protection should also be used when working with high concentrations or large volumes of infectious agents.

Decontaminating bench tops and other surfaces
Bench tops should be wiped with a disinfectant (a phenolic disinfectant, 1% sodium hypochlorite, or 70% alcohol) routinely before and after working with infectious agents or clinical specimens or after spills, splashes, or other contamination by infectious materials. Solutions of disinfectants should be maintained at the work station.

Disposal of contaminated materials
All discarded plates, tubes, clinical samples or other contaminated materials are to be placed in disposal containers at each bench. Special disposal boxes must be used for sharps such as syringes or broken glass to minimize the risk of injury. Avoid overfilling such containers. Containers of contaminated material should be carefully transported to the autoclave room and autoclaved before disposal.

Autoclaving
An autoclave must be available for the BSL-2/3 laboratory and must be operated only by personnel who have been properly trained in its use. To verify that each autoclave is working properly, spore strips or other biological indicators designed to test for efficiency of sterilization should be included in autoclave loads on a regular basis. Each autoclave load should be monitored with temperature-sensitive tape, thermograph, or other means (e.g., biological indicators).

General laboratory policies
All areas of the laboratory must be kept clean and orderly. Dirt, dust, crowding, or clutter is a safety hazard and is not consistent with acceptable biological research. Floors should be kept clean and free of unnecessary clutter. They should be washed with a germicidal solution on a regular basis and after any spills of infectious material have occurred.

Refrigerators and freezers
Refrigerators and freezers should be regularly inspected for the presence of broken vials or tubes containing infectious agents. Wear gloves and proper attire when removing and discarding broken material. Refrigerators and freezers should be regularly cleaned with a disinfectant and defrosted to prevent possible contamination and temperature failure.

Fire prevention
Keep burners away from lamps and flammable materials. Bulk flammable material must be stored in the safety cabinet. Small amounts of these materials, such as ethyl acetate, ethyl alcohol, and methanol, can be stored in safety containers. Turn off burners when not in use. Know the location of fire extinguishers, fire blankets, and showers. Fire safety instructions and evacuation routes should be posted.

If these activities are carried out on a regular basis while performing the functions of the laboratory it greatly reduces the risks of infections. Continuous effort must be made to ensure that no unnecessary risks are taken or hazards ignored. However, in the event of an accident it is prudent to have a reporting mechanism in place to ensure the safety of all the personnel.

GEIS Annual Conference

Elizabeth Njogu

GEIS commitment to the continued education of all its personnel has been shown in many different ways. The organisation of the annual conference is just one of such on going effort to ensure that everything that GEIS undertakes is done with the clear and concise efficiency expected of every one working to make the project a success.

The GEIS Annual Conference has been planned for second year running and the lecture schedule is tentatively planned. Two days of intense training for all the laboratory technicians and clinical officers from all five sites will be carried out following the Microscopy Course in Kisumu in July.

The training will include review of the protocols that are currently running and the processes of patient recruitment and informed consents. There will be a refresher on the SOPs and their implementation as well as outbreak training and more.

It is hoped that the benefits that come out of this conference will help all the people involved solve any problems they face and improve their overall work quality.

IN VITRO EVALUATION OF SEVERAL ANTIMALARIAL DRUG COMBINATIONS AGAINST *PLASMODIUM FALCIPARUM*

Pamela Liyala

Malaria is one of the leading causes of morbidity and mortality worldwide, with over 100 million cases and at least a million deaths a year, most of these deaths occurring in the poorest regions of the world. In the last decades, malaria control and treatment has been complicated by the rapid emergence of resistance to widely used antimalarial drugs such as chloroquine. This has had a dramatic impact especially for the African continent, as few cheap and safe alternative drugs to chloroquine are available (Trape *et al*., 1998; Trape, 2001).

With the rapid emergence and spread of resistance, the output of new antimalarial drugs will not keep pace with the loss of drugs due to resistance. Resistance is the major determinant of a drug's life span. Thus, the primary goal should be to protect the effective use of a drug (Wilairatana *et al*., 2002).

Although the search for a malaria vaccine goes on, there is still some way off before a reliable vaccine can be expected (Whitty, *et al*., 2002). As a result, there is need to develop new and affordable drugs. It is essential to ensure rational deployment of the few remaining effective drugs to maximize their useful therapeutic life while still ensuring that safe, effective and affordable treatment is accessible to those at risk. This requirement has resulted in a re-examination of the potential of combinations of existing products and the development of new combination drugs. A major contribution from scientific research has been the investigation of novel but affordable drugs such as Lapdap, a dapson-chlorproguanil combination (Mutabingwa *et al*., 2001). Combination therapy has been used to delay drug resistance in some drugs for example the combination of atovaquone and proguanil (marketed as Malarone) to combat the rapid emergence of resistance to atovaquone (Canfield *et al*., 1995). Other drug combinations for malaria treatment include Fansimef, the fixed combination of mefloquine, pyrimethamine and sulfadoxine (Bunnag *et al*., 1992) and Coartemether, which is a fixed dose combination of artemether and

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Leptospirosis

Bonventure Juma

Leptospirosis is an infectious disease caused by pathogenic bacteria called leptospires, that are transmitted directly or indirectly from animals to humans hence its characterization as a zoonosis. Leptospirosis is a worldwide infection with a much greater incidence in tropical regions with higher rainfall. The disease is found mainly where humans come into contact with urine of infected animals. It has been identified as one of the emerging or re-emerging infectious diseases. Leptospirosis is a potentially serious but treatable disease.

The disease has short incubation period and its symptoms may mimic those of a number of other unrelated infections such as influenza, meningitis, hepatitis, dengue or viral hemorrhagic fevers. Due to this confusion, Leptospirosis ends up being misdiagnosed by many health care providers translating to 10% mortality in intensive care units and more than a 10% death rate in patients who are not given special medical care on seeking treatment.

A disease presenting as Leptospirosis struck twice in Kenya in July and December 2004 claiming more than 50 lives though the official figures have not been published. Efforts are still in place to confirm if the causative agent was Leptospirosis. Its prevalence and etiology in Kenya is not known so far. The development of simple and rapid assays for diagnosis has been based largely on the recognition that early initiation of antibiotic therapy is important in management of acute disease. The need has also arisen for widely and cheaply available diagnostic assays which can be used more effectively.

Current diagnostic methods for this disease have been: dark field microscopy, ELISA, MAT and PCR. It is advisable that these tests are interpreted alongside patient's presentation, demographic and occupational information. To date, the only reliable diagnostic methods have been based on serology. PCR has been problematic since only a few species have been fully sequenced and the Leptospira genus has many serovars and biovars which have made the process of finding suitable primer sets difficult. Culture methods, while effective are tedious and time consuming.

A number of serological techniques have been employed for its diagnosis with each having its own sensitivity, specificity and predictive values for ⇨

positive and negative. For reliable diagnosis, these techniques have been used together with some success. The Microscopic Agglutination Test (MAT) and Enzyme Linked Immunosorbent Assay (ELISA) are the most reliable methods so far. However, MAT which was developed in 1918 remains the better option for Leptospirosis diagnosis and has a sensitivity if 92%, specificity of 95%, predictive value of a positive (PVP) of 95%, and predicative value of a negative (PVN) of 100%.

The technique is based on cell agglutination and it has been modified considerably since it was first discovered. It detects the antibodies and determines the titer at which agglutination takes place. In addition, the techniques can give an identification of the serogroup to which the infective serovar belongs though it rarely identifies the serovar itself. It detects both IgM and IgG classes of antibodies with ease. The method is also simple and consists of just mixing the test serum with a culture of leptospires and then evaluating the degree of agglutination using a dark-field microscope for identification. According to the Taxonomic Committee of Leptospira, the end point is defined as the dilution of serum with 50% agglutination. The microtiter plates used in this technique are not specified, any 96 flat bottomed wells can do.

Control of the disease in tropics where it is highly endemic is a matter of concern. Vaccination could work but due to the wide range of serovars and biovars, it is difficult to develop a vaccine which would be effective for all the available serovars.

A thorough epidemiological surveillance is highly needed to for this disease in Kenya given that the country lies in the tropics where the disease can thrive. The findings from such a study could provide information on what animals form the major reservoirs for human Leptospirosis in Kenya (rodents, cattle or canines). Such findings will also form a basis for the design and implementation of control measures, preparedness and management of outbreaks of Leptospirosis outbreaks in Kenya and Africa as a whole.

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lumefantrine (Vugt *et al.*, 1999).

It takes many years before newly developed drugs come to the stages of clinical studies and drug registration. The development of alternative combinations seems to be the only way to limit the pace of parasite resistance to chemotherapy. While we are waiting for new antimalarial drugs to be developed, new drug regimens from a combination of currently available drugs remain a promising way of dealing with the challenge of drug resistance.

Because of the urgent need to overcome the problem of antimalarial drug resistance, the objectives of this study were to assess the *in vitro* efficacy of the following antimalarial drug combinations: azithromycin – amodiaquine, azithromycin – artemisinin, tafenoquine – amodiaquine, tafenoquine – artemisinin, amodiaquine – artemisinin, and atovaquone – proguanil (Malarone).

Ten *Plasmodium falciparum* isolates including reference clones D6 and W2 were cultured and tested *in vitro* against the selected drug combinations using a modification of the semi-automated micro-dilution technique. The data was analysed using Oracle Database Software (Data Aspects), which determined the 50% inhibitory concentration (IC_{50}) and the 50% fractional inhibitory concentrations (FIC_{50}) for drugs in fixed combinations (1:1, 1:3, 3:1, 1:4, 4:1, 1:5). The FIC_{50} values were used to plot the isobolograms.

Azithromycin combinations showed an additive activity with a trend towards antagonism. Tafenoquine combinations were synergistic with a trend towards additivity while amodiaquine-artemisinin combination was synergistic. These results suggest that: tafenoquine in combination with amodiaquine, tafenoquine in combination with artemisinin and amodiaquine in combination with artemisinin should be evaluated for malaria treatment in areas of drug resistance.

News Byte

The GEIS Gazette will be altering its publication schedule. We will move from the currently monthly newsletters to quarterly issues. The articles and items will still go through the same editorial process and all the articles collected during the three month period will be published together. It is designed to increase the publication efficiency and allow for a broader range of articles.

From the Editorial Desk

Epidemiology of Diarrhoeal Illness

Paulomi Patel & Elizabeth Njogu

The enteric department undertook the epidemiological study of diarrhoeal illness in Kenya during the month of May. Samples were collected from the five sites in the GEIS network as well as the U.S. Embassy outpatient clinic. We received a total of 147 samples. 41 from Malindi, 55 from Kisumu, 17 from Mumias, 11 from Alupe, 12 from Isiolo and 11 from the American Embassy.

All the samples were tested for diarrhoeal causing parasites namely: *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum*. Bacterial causes of diarrhoea were also tested for and included: Salmonella spp, Shigella spp, Vibrio spp, Campylobacter spp, and pathogenic *E. coli*. For those bacterial isolates recovered further testing was done to determine they serological typing, the virulence factors and their antimicrobial sensitivity patterns,

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Attn: Sheryl Bedno

GEIS Program
USAMRU - Kenya
P.O. Box 606
Nairobi 00621

ADDRESS CORRECTION REQUESTED

SITE MAILING ADDRESS STICKER